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COLIPHAGE AND BACTERIOPHAGE AS INDICATORS OF GROUNDWATER QUALITY IN CANADA

by

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ABSTRACT

Ten raw urban well water samples and twelve water samples collected from distribution lines after the well waters were treated were examined for bacteriological and coliphage/bacteriophage populations. The raw well waters were found to contain <1/100 mL total coliforms and fecal streptococci, but they all contained varying concentration of coliphage and bacteriophage. The treated waters all were found to have <1 total coliforms and fecal streptococci/100 mL with the exception of one treated water sample from Community C. However, even though the treated water samples contained free and total chlorine levels varying from 0.05 to 1.5 ppm, they all were found to contain usually greater amounts of coliphage and bacteriophage than the raw well waters. Details of the study and implications are discussed.

RÉSUMÉ

Dix échantillons d'eaux brutes provenant de puits urbains et douze échantillons d'eau provenant du réseau de distribution (eau traitée) ont été soumis au dépistage des populations bactériennes et de coliphages/bactériophages. Les eaux brutes renfermaient < 1/100 mL de coliformes et de streptocoques fécaux au total mais présentaient des concentrations variables de coliphages et de bactériophages. Les échantillons d'eau traitée renfermaient tous < 1/100 mL de coliformes et streptocoques fécaux au total, à l'exception d'un échantillon provenant de la municipalité C. Cependant, même si les échantillons d'eau traitée renfermaient du chlore libre et du chlore total en teneurs variant de 0,05 à 1,5 ppm, ils contenaient tous des quantités de coliphages et de bactériophages généralement plus élevées que les eaux brutes. Les détails de l'étude sont présentés et les implications des résultats sont traités.

MANAGEMENT PERSPECTIVE

Recent Canadian studies have shown the presence of coliphage and bacteriophage in coliform free treated drinking water whose source was surface waters from lakes and rivers. In this study, we report on the finding of coliphage and bacteriophage in urban well waters, both before and after treatment. In all cases, no total coliforms were isolated. Thus groundwater which was believed to be one of our last frontiers of easily accessible pure water supplies, have now been found to be microbiologically contaminated. Furthermore, the treatment accorded these drinking waters seems insufficient to remove the coliphage/bacteriophages.

The presence of coliphage/bacteriophages in these well waters and drinking waters, without coliform presence, strongly suggests (a) that human enteric viruses can also survive the normal treatment and disinfection processes given to these waters; (b) that coliform free drinking waters are not necessarily pathogen-free waters; and (c) drinking waters containing coliphage and bacteriophage should be thoroughly investigated for the presence of human enteric viruses. The results of the study are strongly supportive of the recommendation that the coliphage test should be included as part of any potable water testing scheme to enhance consumer protection.

PERSPECTIVE - GESTION

Des études canadiennes récentes ont révélé la présence de coliphages et de bactériophages dans des eaux potables traitées, libres de coliformes, provenant de lacs et de cours d'eau. Dans la présente étude, les auteurs rapportent qu'ils ont trouvé des coliphages et des bactériophages dans des eaux provenant de puits urbains, à la fois dans les eaux brutes et dans les eaux traitées. Dans aucun cas, les auteurs n'ont pu isoler de coliformes totaux. Les eaux souterraines, que nous pensions être notre dernière source d'approvisionnement en eau pure et facilement accessible, seraient donc contaminées par des microorganismes. De plus, le traitement donné à ces eaux potables semble insuffisant pour faire disparaître les coliphages/ bactériophages.

La présence de coliphages/bactériophages dans les eaux provenant de puits et dans l'eau potable ne renfermant pas de coliformes implique : a) que les entérovirus peuvent également survivre à la désinfection et au traitement normaux que subissent ces eaux; b) que l'eau potable ne renfermant pas de coliforme n'est pas nécessairement une eau libre d'agents pathogènes et c) que l'eau potable renfermant des coliphages et des bactériophages devrait être examinée attentivement pour déceler la présence d'entérovirus. Les auteurs de la présente étude sont donc tout à fait d'accord avec la recommandation selon laquelle le test permettant de déceler les coliphages devrait faire partie de tout programme d'échantillonnage de l'eau potable, de manière à assurer la protection du consommateur.

INTRODUCTION

With the increasing world wide industrialization and urbanization over the past 30 years, and with the concomitant higher demand for chemicals and water supplies, both the developed and developing nations face increasing ecological problems from the release of domestic and industrial wastes to the environment. One of the last frontiers of easily accessible pure water supplies, for our rapidly urbanizing world, is ground water, and even these waters and aquifers are being rapidly contaminated by a variety of chemicals and occasionally microorganisms.

Recent microbiological studies in developing and developed countries are indicating the increasing presence of coliphage/bacteriophage in treated drinking water, both tap and bottled. In studies by Grabow (1986) and Kott et al. (1978), it has been shown that coliphage/bacteriophage and viruses are similarly resistant to chlorination and both are more resistant than E. coli. Therefore, the presence of coliphage/bacteriophage in potable waters is an indication of inefficient disinfection and possibly the presence of human enteric viruses in these waters. In some countries (Petrovicova et al., 1988) the presence of coliphage in potable water supplies is a signal for the testing of these waters for human enteric viruses.

As the technology for the testing of coliphages and bacteriophages (Havelaar, 1986; Wentsel et al., 1982 and Dutka et al., 1987) has advanced to the stage where it can be easily performed by routine

water quality laboratories, it was decided to use this technology to evaluate the virus-free nature of representative ground water supplies in the province of Ontario. The results of this preliminary survey are described.

METHODS

Water Samples

Twenty-two water samples were collected from raw ground waters and treated ground water, drinking waters from three different urban areas in the province of Ontario, over a two month period. Twelve samples were taken from distribution lines after treatment. The remaining ten samples were raw waters taken immediately after they were pumped out of the wells and prior to any disinfection treatment. The treated samples were dechlorinated with sodium thiosulphate (APHA, 1985) and maintained at cool temperatures until processing was completed within a maximum of 4 hr of collection.

Microbiological Tests

All water samples were subjected to the following microbiological population estimation techniques: total coliforms by membrane filtration procedures using Gelman GN6 membranes LES Endo agar with 24 hr incubation at 35°C, fecal coliforms by membrane filtration procedures using m-TEC agar with 24 hr incubation at 44.5°C; fecal streptococci

by membrane filtration procedures using m-Enterococcus agar with incubation at 35°C for 48 hours, and standard plate count using membrane filtration procedures and a modified SPC agar with incubation at 35°C for 48 hours. Details of the microbiological procedures can be found in the M.O.E. manual HAMES (1984).

Coliphage and Bacteriophage Tests

The procedures detailed by Havelaar (1986) for enumerating bacteriophage from natural water samples were used in this study. Basically 5 litres of raw water and 10 litres of treated water for each host used, was filtered through an electropositive filter at pH 6.0 in the presence of 0.005 M imidozole buffer. Elution was by a solution of beef extract at 10 g/L, pH 7.0 with 0.1M Mg^{2+}. This method was found to underestimate bacteriophage populations by at least 30-50%. Four bacterial hosts were used in this study, two for coliphage and two for F+ specific (RNA) bacteriophage detection. The *E. coli* hosts were *E. coli* C, and *E. coli* HrfH. The *S. typhimurium* hosts were WG49 specific for F(+) RNA bacteriophage and WG45 for F-somatic bacteriophage (Havelaar, 1986).

Chemical Tests

The Ontario Ministry of Environment procedures were used to test for total and free Cl_2 (C.P.W.S, 1987).

RESULTS AND DISCUSSION

Examples of typical raw and treated ground water results for the three urban areas are shown in Table 1. No total coliform, fecal coliform or fecal streptococci data are shown as all raw well waters and treated well waters indicated <1 organisms per 100 mL with the exception of treated water from well C, which contained 1 fecal streptococcus per 100 mL. It can be seen that in all samples presented, as well as those not shown, coliphage and bacteriophage were present before treatment as well as after disinfection. It should also be noted that the coliphage and bacteriophage levels after treatment were usually higher than the raw source waters and only rarely equivalent. This may be due to a shedding of coliphage/bacteriophage from overstrained sand filters and insufficient chlorine or inadequate chlorine contact time.

A striking feature of these data was the consistent recovery of all four different types of bacteriophage even when total and free chlorine levels were as high as 1.50 and 1.30 ppm.

Grabow in 1968 described many studies that indicated that most common pathogenic viruses are more resistant to chlorination than *E. coli*. Kott *et al*. (1978) has shown that coliphages are similarly resistant to chlorination as polio viruses. Furthermore, Simkova and Cervenka (1981) reported that "observations on coliphages appear to give a good indication of polio virus survival in a given water system; but further verification is needed to confirm this relationship in respect to other enteroviruses".

Studies of drinking water by Petrovicova et al. (1988) have shown similar results in Czechoslovakian waters and they state "presence of coliphages in drinking water is an indication for aimed virological examination". Furthermore, since 1987, the coliphage enumeration procedure as a means of controlling water quality has been adopted by the Hygiene Services and Water Supply Authorities in Slovakia (Petrovicova et al., 1988).

Thus, the presence of coliphage and bacteriophage in these drinking water samples, without coliform presence, strongly suggests (a) that human enteric viruses can also survive the normal treatment and disinfection processes given to these potable water samples; (b) that coliform-free potable waters are not necessarily pathogen-free waters, and (c) drinking waters containing coliphage and bacteriophage should be thoroughly investigated for the presence of human enteric viruses.

The observations of coliphage and bacteriophage of E. coli and S. typhimurium, two pathogens and enteric organisms, in the raw well waters is disturbing. Well waters, especially deep wells, are usually considered to be free of sewage or pathogenic organisms. In this study, no total or fecal coliforms or fecal streptococci were found in 100 mL portions of the raw waters, and yet bacteriophage specific to E. coli and S. typhimurium were isolated and enumerated not rarely, but in every sample. The sources of these coliphage/bacteriophage are unknown, and their survival through the treatment/disinfection procedures may be indicative of an unsuspected infecting source.

In conclusion, we believe, on the basis of these Canadian data and similar international data (Palmateer et al., 1989; El-Abagy et al., 1988; Ratto et al., 1989; Sim and Dutka, 1987, and Simkova and Cervenka, 1981) that the coliphage test merits further study as an indicator of insufficient or inefficient disinfection procedures and potential viral survival.

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Table 1. Coliphage and bacteriophage presence in raw and treated drinking water from wells servicing three urban communities.

	Com.[6]A June 1	Com. B June 4	Com. C June 9	Com. B June 19	Com. C June 24	Com. C June 30	Com. C July 14
ECC/L[1]	1.9	-	2.5	-	6.3	25.0	26.7
ECHFR/L[2]	4.1	-	12.5	-	11.3	20.0	31.7
WG49/L[3]	9.1	-	8.3	-	23.8	53.3	36.7
WG45/L[4]	5.6	-	2.0	-	16.3	16.7	16.7
SPC/100 mL	1,300,000	-	800,000	-	400	20	1100
	Com. A June 1	Com. B June 4	Com. C June 9	Com. B June 19	Com. C June 24	Com. C June 30	Com. C July 14
ECC/L[1]	1.9	10.0	4.5	2.5	17.5	35.0	28.6
ECHFR/L[2]	4.1	1.3	6.5	1.7	45.0	15.0	59.0
WG49/L[3]	6.6	4.0	6.0	26.7	45.0	92.5	30.0
WG45/L[4]	6.3	2.0	4.5	5.0	32.5	47.5	23.3
SPC/100 mL	200	-	600	-	2400	0	2500
Free Cl_2 (ppm)	-[5]	1.3	0.05	-	0.80	0.30	0.50
Total Cl_2 (ppm)	-	1.5	0.10	-	0.90	0.80	0.60

[1]E. coli C coliphage
[2]E. coli HfrH coliphage
[3]F^+ specific bacteriophage (RNA)
[4]F^- somatic bacteriophage
[5]not done
[6]Com. = Community